

## ABSTRACT

The present invention is to provide a multiple detection method that can detect contaminating microorganisms existing in foods, including pathogenic *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella* spp., with high sensitivity comparable or even superior to official methods, comprising the steps of amplifying a plural number of target genes with a single PCR reaction tube and analyzing the same. The following steps are performed consecutively: (A) a step of extracting DNA of the target microorganisms to be detected by treating with at least a lytic enzyme such as Achromopepidase and Lyzocyme and/or bacteriocin having lytic activity such as Enterolysine, a surfactant and a protein denaturing agent; and (B) a step of mixing a specific primer to the target microorganisms to be detected to perform multiplex PCR. Further, it is preferable to add a step of culturing with a culture condition where 1 CFU/100 g microorganisms becomes  $10^3$  CFU/ml or more after 18 to 48 h of culture, for example that the pH after culture becomes 5.1 or more, before the step of extracting DNA of the target microorganisms to be detected.